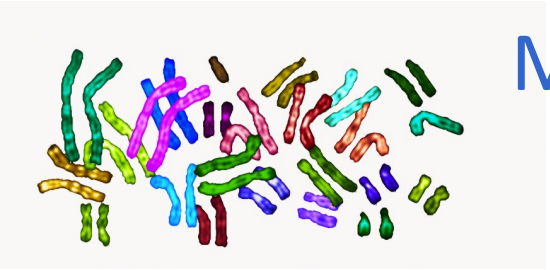

Molecular Pathology in Neuro-oncology

Over the last 10 years, we have seen a number of interesting developments in molecular pathology impact on clinical practice. Molecular Pathology is being used for both the diagnosis, and treatment stratification of brain tumours. This overview highlights some of the important innovations for Gliomas.

WHO classification

The WHO classification of brain tumours focuses on the architecture of tumours visualised at low power using H&E staining. Gross morphology can be used to establish diagnosis in well differentiated tumours. The addition of molecular pathology can provide useful insight into the histogenesis of poorly differentiated tumours, and tumours that are heterogeneous at the cellular level. Glioblastoma, which is the commonest malignant tumour we encounter in our clinic, is an example of an extremely heterogeneous tumour.

Gene expression analysis of Glioblastoma

Hans Scherer at work. His thinking was way ahead of his time, and he was the first to suggest that Gliomas spread by infiltration into surrounding normal brain tissue.

Hans Scherer was a German neuropathologist working in exile in Belgium during the Second World War. He observed two distinct patterns of glioblastoma. Primary GBM presented in older patients with short clinical duration (weeks to months), against Secondary GBM which presented in younger patients who were previously diagnosed with lower grade astrocytomas. These patients typically presented having developed symptoms of seizures or other neurological deficit over a much longer period of duration. It is impressive that he established this purely from biopsy and necropsy material, prior to the advent of CT and MRI scanning.

Despite the similar histological features of primary and secondary GBM, molecular pathology started to demonstrate that the tumours displayed quite distinct patterns of gene expression. The initial approach was 'targeted' in that pathologists looked for the presence or absence of gene mutations that had been characterised in a range of other tumour types. Primary glioblastomas typically show EGFR overexpression, PTEN (MMAC1) mutations, CDKN2A (p16) deletions, and less frequently, MDM2 amplification. Secondary glioblastomas often contain TP53 mutations as the earliest detectable alteration. This level of characterisation was state of the art in the late 1990's, but had very little impact on clinical practice.

Karyotype analysis of malignant gliomas

In the mid 1990's, similar attempts to classify gliomas were being performed using chromosomal analysis. Fluoresence in-situ hybridisation (FISH) and Comparative Genome Hybridisation (CGH) techniques were used to localise some of the commonest chromosomal deletions observed in malignant glioma. The presence or absence of these deletions was then correlated to the histological grade of tumour, and patient outcome. The results were very interesting. It was observed that deletion on the short arm of chromosome 1 (1p.36) and the long arm of chromosome 19 (19q13.3) were commonly observed in around three quarters of all oligodendrogliomas and 20% of astrocytomas.

In 1998 It was also observed that combined deletions of both of these regions were observed in patients with oligodendrogliomas, and were strongly associated with prolonged survival in patients with Grade III anaplastic oligodendrogliomas, and mixed oligoastrocytomas What is fascinating is that co-deletions of 1p and 19q are found in both low grade oligodendrogliomas (where the prognostic significance is less clearly established), and high grade gliomas (where they are associated with a better prognosis and greater sensitivity to chemotherapy treatment). The prognostic significance of 1p19q co-deletion has now been established clearly in prospective clinical studies (methodologically the best study demonstrating this was the RTOG 9402 study).

The Cancer Genome Atlas and the era of genomic analysis

In 2006, the National Cancer Institute piloted a project to collect data on the complete genome sequence from a range of human tumours. Glioblastoma was one of the tumours to be included in the pilot, which is now expanded to include 25 common tumour types. The project was called the Cancer Genome Atlas. Full genome sequence data is available for over 600 patients in some of the tumour types. In addition to the gene sequence data, the atlas contained information on the outcome of the patients, how long they survived and how they responded to treatment. With this wealth of data it becomes possible to perform pattern analysis using novel statistical techniques, to look for clusters of genes that associate with different patterns of clinical behaviour. In 2010 RoelVerhaak's group from the MD Anderson Cancer Centre performed such an analysis for GBM and came up with 4 distinct tumour phenotypes which expressed similar patterns of gene expression, namely classical, neural, mesenchymal and proneural. The results are simplified in Table 1.

Subtype	Genetic hallmarks	Clinical features
Classical	Increased EGFR expression, CDKN2A deletion TP53 mutations uncommon	This subgroup survives the longest in response to aggressive treatment.
Mesenchymal	Frequent NF1 deletions Frequent PTEN mutations	Significant survival responses to aggressive treatment
Proneural	Mutations in PDGFR and IDH1 Association with 1p19q co-deletion	Commoner in younger patients, trend towards longer overall survival, though aggressive treatment is not associated with improved survival.
Neural	Expression of neurone markers	Occurred in older patients, response to aggressive therapy is not as marked as for Classical and Mesenchymal groups.

Table 1 : Classification of Glioblastoma into four genetically distinct subtypes on the basis of genomic analysis.

So is this the 'Holy Grail' for understanding the molecular characterisation of Glioblastoma. Well not quite! The Verhaak classification is interesting in providing proof of concept that you can divide tumours by genetic fingerprint. However, the total number of patients included in the analysis (n=201) and the number of genes (n=601) is still small. Furthermore, the quality of the clinical data and treatment details (radiotherapy, chemo, surgery) is very poor compared to current standards. An integrated approach to repeat and validate this study is needed and it would not be appropriate to base clinical decisions on this analysis just yet.

Metabolomics

As we move towards a systems biology approach to understanding cancer, the focus has moved from understanding gene expression, to understanding the effects of gene expression in the cell in terms of protein expression (proteomics) and metabolic changes (metabolism). It is ironic how things have come full circle as Otto Warburg demonstrated in the 1940 that altered metabolism and increased glycolysis were hallmarks of cancer cells, in order to support increased rates of proliferation. Move forward 70 years, and interest has focused on isocitrate dehydrogenase enzymes, and their role in the 'metabolic reprogramming' of gliomas. When functioning normally IDH1 catalyses the conversion of isocitrate to alpha-ketoglutarate. This produces NADPH as part of the Krebs cycle. Tumour cells also convert Glutamate into citrate outside of the Krebs cycle. Mutations of IDH1 lead to the accumulation of 2-hydroxyglutarate (2HG) in cells. This causes activation of hypoxia pathways, mainly HIF1 Alpha, and results in increased expression of VEGF by the tumour cell. In addition, 2HG has an effect on histone methylation, which is important for the regulation of gene expression in the cell. The effect is a loss of control of gene expression in the tumour cell. (See Figure 2)

Clinical studies (EORTC 26951) have demonstrated that many Anaplastic Oligodendrogliomas and mixed anaplastic oligoastrocytomas bear mutations of IDH1. This has been shown to confer a distinct survival advantage, whilst not predicting a response to chemotherapy treatment.

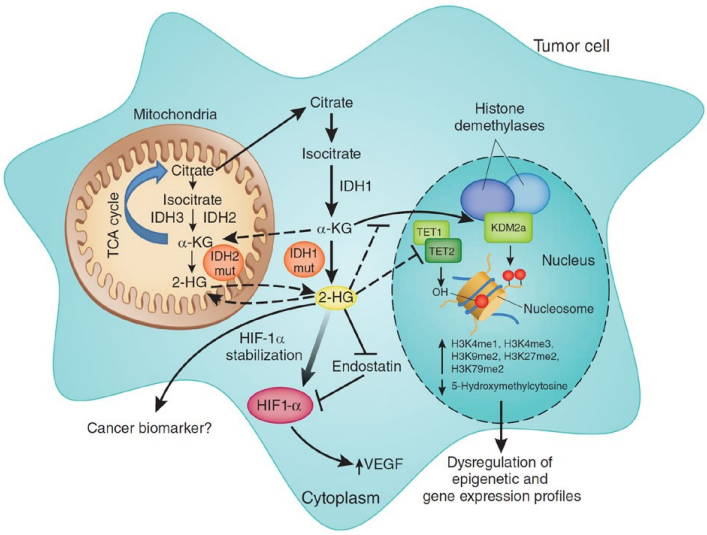

Figure 2.Effects of IDH1 mutations and accumulation of 2 hydroxyglutarate in tumour cells. From: Prensner & Chinnaiyan. Metabolism Unhinged: IDH mutations in cancer. Nature Medicine 17 (2001) 291-293

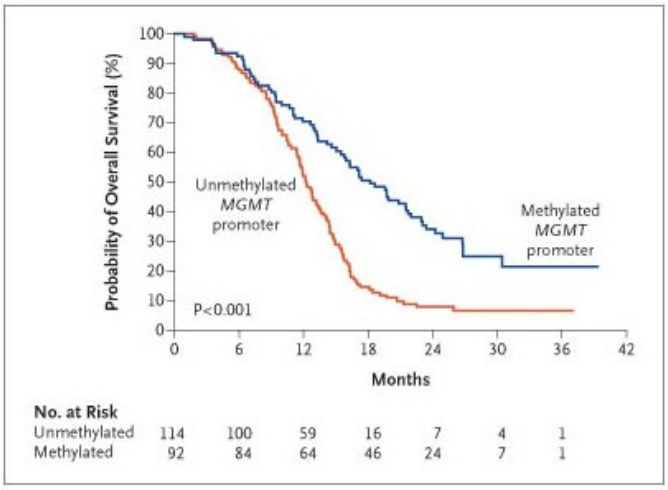

Figure 3. Overall survival of GBM patients is strongly correlated with tumour MGMT promoter methylation status. From Hegi at el NEJM 2005.

The MGMT story

Perhaps the most successful molecular pathology assessment in Glioblastoma is that of MGMT (O6-methylguanine–DNA methyltransferase). The MGMT gene encodes a repair protein responsible for excision of alkylated DNA bases, which are the main effect of Temozolomide chemotherapy. A functioning MGMT pathway will therefore lead to resitance to the effect of Temozolomide. However, in a proportion of patients, the repair gene is silenced by methylation of its promoter sequence. In these patients, the tumour is susceptible to the effects of alkylating agentssch as temozolomide. In a seminal paper in the New England Journal in 2005, Monika Hegi performed a survival analysis using data from a large EORTC study of chemoradiation treatment in Glioblastoma. She observed that MGMT methylation status was strongly prognostic of overall survival for patients treated with Temozolomide and radiation (See Figure 3)

Summary

This short overview shows how genetic analysis of gliomas is revealing more insight into the evolution of tumours, and increasingly, we will see the use of molecular pathology to guide treatment decisions and predict response to therapy.

If you see a patient with a Glioma in clinic today, have a look at the information available on the pathology form, and try and see how this information is used by the team in the decision making process.